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Soft-Tissue Effects of the Holmium:YAG Laser: An Ultrastructural Study on Oral Mucosa

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Background and Objective: **The specifics of the ablation mechanism of the holmium:YAG laser remain largely unexplored. Following laser exposure to the oral mucosa of rats, the ultrastructural damage profile obtaining to varying degrees in blood vessels, erythrocytes, nerves, and muscle cells was examined. An attempt was made to relate the cytoplasmatic alterations to the tissue ablation modes of midinfrared lasers described in the literature.**
Study Design/Materials and Methods: **The biological effects of a new pulsed holmium:YAG laser (λ = 2,120 nm) on the oral mucosa of rats were examined by light and transmission electron microscopy. Laser incisions reaching into the muscle layer were made on different sites of the tongue of white rats. Laser energy (400 mJ, 2.5 μs pulse, 2 Hz) was delivered to the target via 400 μm nylon fibers.**
Results: **The fine-structural morphology of the sublingual mucosa after laser surgery of the epithelial surface revealed no carbonization layer but a 150-μm-wide zone of lacunar structures extending to the lamina propria. In the muscle cells there is partial decomposition of the cell contents resulting in the development of electron optically empty spaces within the cortical cytoplasm underneath the intact plasma membrane of the muscle cell. The organelles within the cell remain ultrastructurally intact.**
Conclusion: **These features support the assumption of an additional nonthermal holmium:YAG laser-tissue interaction. Lasers Surg. Medicine 20:265–271, 1997.**

Key words: Hol:YAG laser surgery; mucous membrane; ultrastructure; photoablation; infrared laser

INTRODUCTION

Infrared lasers (CO_2, Nd:YAG) commonly employed for microsurgery of the aero-digestive tract act on tissues by thermal ablation and produce extensive areas of damage. In contrast, pulsed solid-state crystal, midinfrared lasers operating at wavelengths from 1,980 nm to 2,940 nm and UV excimer lasers affect biological tissue by a nonthermal mechanism that remains largely unexplored. The action of these lasers is mainly based on the nonlinear process of photoablation

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and plasma-mediated photodisruption, thus permitting precise tissue removal with only minimal thermal damage to surrounding tissues [1, 2]. The combination of high-peak power output and short pulse duration in erbium:YAG and holmium:YAG laser systems has been hypothesized to allow precise energy delivery to the tissues with little associated thermal damage [3]. These supposedly photothermal laser-tissue interactions reach optimum efficacy with the specific water absorption coefficient for radiation in the midinfrared. On the water absorption curve, the maximum is at 2,940 nm, closely matching the emission wavelength of the erbium:YAG laser [4]. At this wavelength, however, limitations exist with regard to suitable fiber-optic delivery systems, and the usefulness of erbium laser irradiation in intracorporal endoscopic application is severely limited [5]. A secondary peak of the water absorption curve occurs at ~1,950 nm. The holmium:YAG laser has an emission band at a wavelength of 2,120 nm, which can be readily transmitted by optical fibers with only coupling losses. In addition, the holmium:YAG laser offers good control and predictable thermal energy transfer to the target tissue. This laser has therefore been put to clinical use during functional endoscopic endonasal surgery in patients with chronic sinusitis or with nasopharyngeal carcinoma [6, 7].

Previous experimental studies on test cuts in different tissues in vitro and in vivo have shown that 2,940 nm and 2,120 nm radiation can effectively cut a variety of tissues, especially bone, leaving between 5 and 600 μm of damage [8–11]. The presence of plasma illumination during hard- and soft-tissue processing with high fluences suggests that thermal ionization or photoablation occurs. However, light microscopic changes following laser surgery are not specific for photoablation but are in part also consistent with a simple thermal damage profile of laser energy with very high water absorption. These findings are particularly salient with higher repetition rates using the new holmium:YAG infrared and 308 nm UV excimer laser systems [12]. More recent studies involving the holmium, erbium, and thulium YAG lasers either support a thermally mediated explosive pyrolysis of substrate or suggest that plasma-mediated photodisruptions and photoacoustic effects may also play a role in the destruction of substrate [13–16]. Scanning electron microscopy of human biological tissues treated with laser wavelengths in the midinfrared in several clinical specialities, including ophthalmology, dermatology, and dentistry, support a photothermal mechanism of laser-tissue interaction [17, 18]. Nevertheless, there is still a lack of research findings on the ablation of soft tissues and mucous membranes in live animals [19, 20]. In the present study the biological effects of a new pulsed holmium:YAG laser on the oral mucosa of rats are examined by electron microscopy. Unlike other reports on holmium:YAG laser–tissue interaction our study correlates histological findings and physical effects on the basis of cytoplasmatic changes, which are in part attributed to photothermal or photoablative effects. The physical settings of the laser equipment are described with regard to their importance as basic determinants of photoablation achieved with the use of flexible light-conducting fibers.

MATERIALS AND METHODS

Animal Model

Scientific protocol design, animal welfare, and conditions for animal use were approved by the Institutional Animal Care and Use Committee of the University of Vienna under the supervision of the Ministry of Science and Research. Twenty male white rats (Sprectalii OFA), 4–5 months of age, were anesthetized intraperitoneally with Pentobarbital sodium (30 mg/kg body weight) and underwent laser surgery of the tongue under sterile operating room conditions. The sublingual side of the tongue, being a muscle body covered with mucosa, was used as a model for the vocal cord. The operative aim consisted in an incision extending through the mucosa into the muscle layer. The animals were sacrificed immediately after laser application, and the tongues were resected for histologic examination.

Laser Light Delivery System

Laser surgery was performed with a pulsed holmium:YAG laser at a wavelength of 2,120 nm. The 2.5 μs laser pulse of the holmium:YAG laser was transmitted to the target site by nylon fibers with a core diameter of 400 μm. All fibers used had a length of 300 cm. On the sublingual side of the tongue, two incisions of 4 mm length were made 2 mm away from and parallel to the midline of the tongue. In order to achieve photoablation, the tip of the nylon fiber was placed at a distance of 1 mm from the surface of the tongue and guided by an apparatus ensuring a continuous reproducible laser incision (stepper motor). The holmium:YAG laser was used at a setting that seemed clin-

ically relevant on the basis of pilot studies carried out by our study group on soft tissues with various energy and pulse duration settings (unpublished data). The laser was tested in the following setting: power 500 mJ (at 680 V), repetition rate 2 Hz, pulse duration 2.5 μs, exposure time 10 s.

Pulse Energy Analysis

The pulse energy delivered from the holmium:YAG laser was measured with a pyroelectric energy detector (Gentec ED-500, Dallas, TX) connected to an oscilloscope (Tectronix, Vancouver, Canada) and a thermal power monitor (Gentec TPM).

Histology

The animals were sacrificed by an overdose of Nembutal and the tongues were harvested immediately. For light microscopy specimens were fixed in 5% formalin, embedded in parablast, and stained with hematoxilin-eosin. For transmission electron microscopy, specimens were fixed in a paraformaldehyde glutaraldehyde solution. Upon rinsing in 0.1 M sodium cacodylate buffer (pH 7.4, 12^h, 4°C) the specimens were postfixed in 3% OsO_4 in distilled water for 1½ hours at 0–4°C. Subsequently they were dehydrated through an extended graded ethanol series and embedded in Epon 812 and ultra-thin sections (30–50 nm) were made and were contrasted with uranyl acetate and lead citrate. Sections were obtained using an Ultracut E microtome (Reichert and Jung, Austria). Sections were examined and photographed in Jeol JEM-1200 EX electron microscope operated at 80kV.

RESULTS

The examination of the gross and histologic effects of in vivo holmium:YAG laser ablation on the oral mucosa of rats revealed no tissue contraction during laser surgery and no macroscopic swelling of the tongue. Hemostasis was sufficient and the repetition rate of 2 Hz permitted good control of the depth of laser penetration. A superficial scab was seen as a result of heat effects.

Laser Light Delivery System: Pulse Energy Analysis

The holmium:YAG laser output in our setting was 500 mJ measured directly at the laser coupler. The pulse energy delivered at a distance of 1 mm from the fiber tip was 400 mJ. The laser spot at the target had a diameter of 400 μm. The holmium:YAG laser beam also produced an audible crack and a 1 mm long yellow-orange flame on the target site at the 500 mJ exposure setting because of photoacoustic-related effects.

Histology

No zone of carbonized tissue was seen in any sample of the histologic specimens. Histologic study revealed tissue defects with central V-shape craters reaching into the muscle layer. Detachment of the surface epithelial layer was seen in the marginal area of the laser target zone. The area of coagulative necrosis measured at 370–640 μm (Fig. 1).

The fine-structural morphology of the sublingual mucosa after laser surgery on the epithelial surface was examined by electron microscopy. There was no carbonization layer but a 150-μm-wide zone of lacunar structures that extends to the lamina propria. The blasted amorphous cells are dissociated by vacuoles and parts of the basal lamina are split up (Fig. 2). The blood vessels show endothelial damage of varying degrees (Fig. 3). Within the lumen there is increasing vacuolization of the erythrocytes, ultimately leading to the formation of membrane ghost erythrocytes. The nerves displayed different degrees of damage of the myelin sheets and of the axoplasms; occasionally, concentric myelin bodies occurred (Figs. 4, 5). In the muscle cells there is partial decomposition of the cell contents resulting in the development of electron optically empty spaces within the cortical cytoplasm underneath the intact plasma membrane of the muscle cell (Fig. 6).

DISCUSSION

The specifics of the ablation mechanism of the holmium:YAG laser remain largely unexplored. It is assumed that similar to the mechanisms of bone ablation and soft-tissue ablation proposed, respectively, by Nelson at al. [21] and Walsh et al. [14] with the use of the erbium:YAG laser, holmium:YAG laser light is effectively absorbed in tissue by water and causes rapid heating of a small volume. This results in high internal pressure that may lead to material removal in the form of a microexplosion with parts of the cellular elements being ejected as microscopic particles. This mode of ablation is not unique to 2,120 nm radiation [22–24]. The suggestion that gases of high temperature and pressure developed at the site of absorption may be capable of causing tissue disruption is consistent with our ultra-

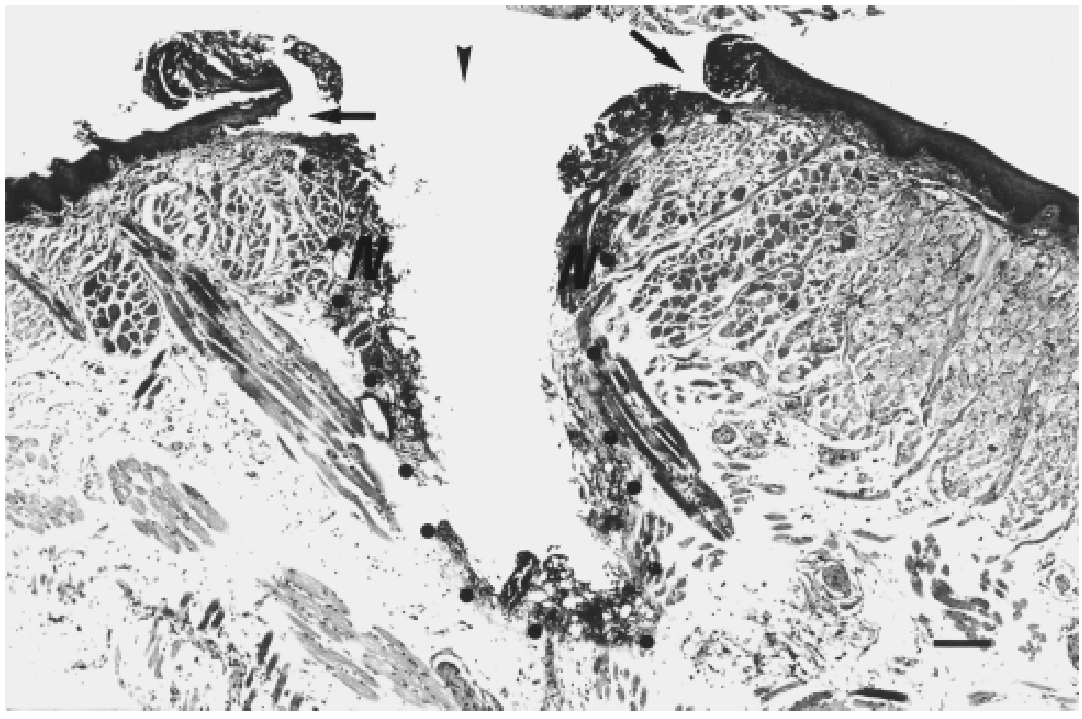

Fig. 1. Light photomicrograph (× 40) of a holmium:YAG laser incision on the sublingual side of a rat tongue reaching into the muscle layer in wedge-like form (arrowhead); arrows indicate the detachment of the surface epithelium in the margin area of the laser target zone. Borderline between necrosis zone (N) and muscle layer marked with dots. Bar = 100 μm.

structural findings: vacuoles are formed, probably filled with CO_2 or O_2, and parts of the basal lamina are split up in the border zone of an evaporization crater.

The micromorphologic damage profile of the blood vessels and the formation of myelin bodies in the nerves as a sign of damage to phospholipids can be related to a photothermal laser-tissue interaction. In a scanning electron microscopic study, Hill et al. [17] demonstrated blast effects and tissue shredding of trabecular meshwork following holmium:YAG exposure as well as the development of multiloaded steam bubbles in aqueous environments. In addition, high-speed photographs of the ablation process have revealed that explosive removal of tissue occurs [25, 26]. Tissue interaction studies on nonskeletal tissues using laser systems operating in the midinfrared have shown that the extent of thermal tissue damage varies according to the type of tissue [11, 18] and increases with higher repetition rates.

Histologic findings reported by our study group as well as other investigators show that the area of tissue damage extends around the target zone with nearly constant thickness. A purely thermal ablative effect should affect the side

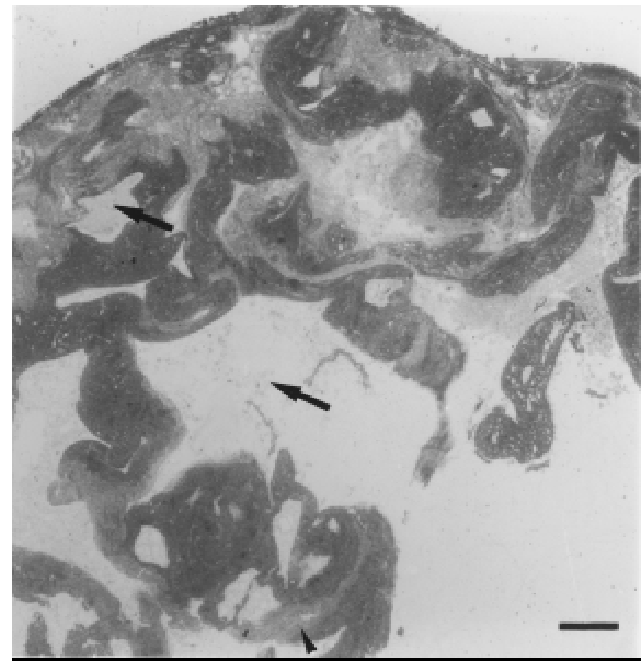

Fig. 2. Electron photomicrograph of the marginal zone of holmium:YAG laser damage with blasted amorphous cells dissociated by vacuoles (arrows) and parts of the basal lamina split up (arrowhead). Bar = 500 nm.

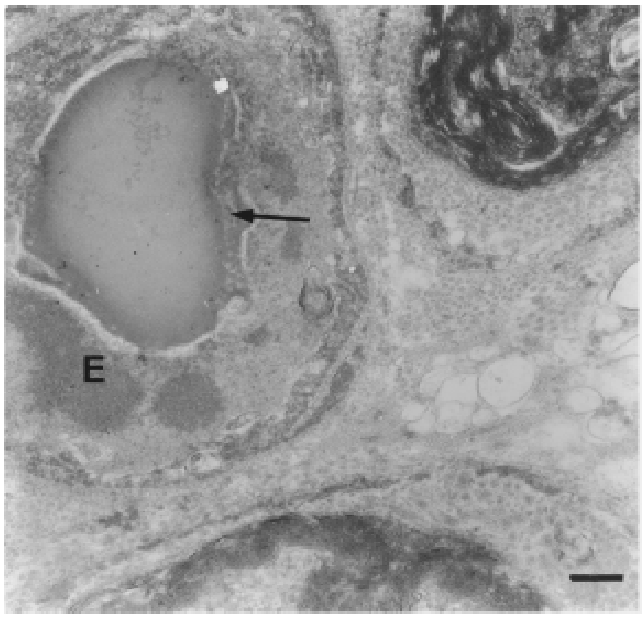

Fig. 3. Electron photomicrograph showing a capillary with swollen endothelium (E); within the lumen a damaged erythrocyte with erratically contoured cell membrane (arrow). Bar = 500 mm.

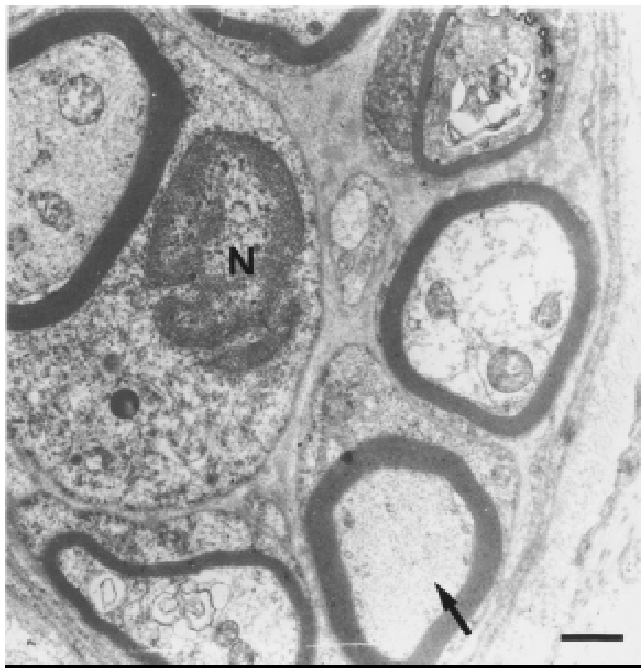

Fig. 5. Nerve with varied stained axoplasms showing different degrees of loss of cell organelles (arrow); nucleus (N). Electron photomicrograph bar = 1 μm.

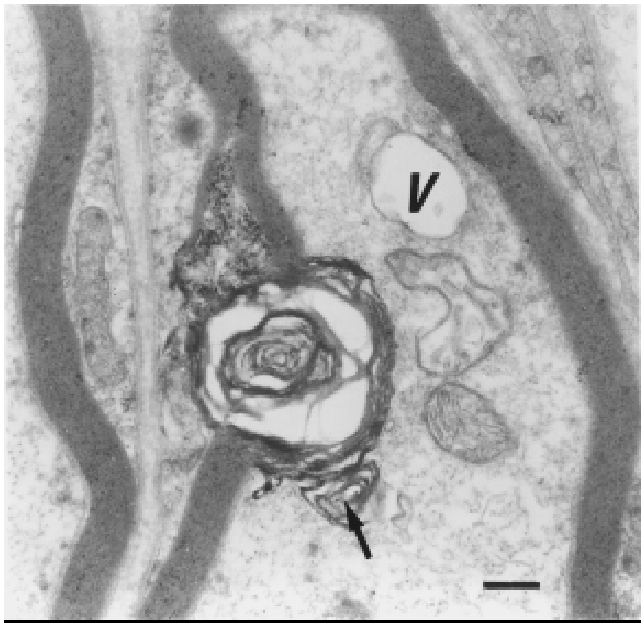

Fig. 4. Electron photomicrograph demonstrating the damage profile of mucosal nerves with interrupted myelin sheets, and occurrence of myelin bodies (arrow); axoplasm with vacuoles (V); appearance of electron optically empty spaces with partial loss of cell organelles. Bar = 200 nm.

walls less and should not be influenced by the number of laser pulses. Yet, if vapor dissipation is hindered by a rapid succession of pulses, heat can also dissipate to the side walls due to the longer tissue-laser energy interaction time. Therefore, damage areas become larger as the pulse frequency of the laser increases. The characteristics of a high-energy, ultrashort-pulsed laser beam may further complicate the effect of the holmium: YAG beam as it interacts with tissue. In our view this type of laser cannot only produce a photothermal effect, but, due to the pulsing of the beam, may also produce a photoablative and a photoacoustic effect.

Intense laser radiation can induce plasma formation via a number of mechanisms. Plasma formation can be achieved not only by rapid heating of absorbing materials (pulse duration in the range of microseconds) to temperatures of several thousand degrees Celsius, but also by optical breakdown. Photoablation is generated if an irradiance in the range of 0.1–10 J/cm^2 and pulse durations in the range of no more than 3 μs can be achieved. This phenomenon was first described by Srinivasan in 1986 [2]. The major part of the incident energy is consumed in the ablative process, and only a small fraction of the energy remains in the tissue as thermal energy. Therefore, ablation of the exposed mucous membrane can be performed more precisely. The laser energy generated in our experimental and clinical settings ful-

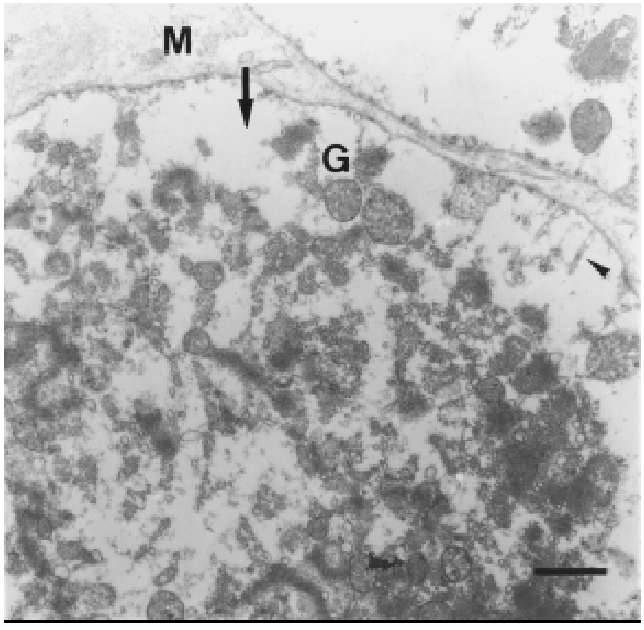

Fig. 6. Electron photomicrograph demonstrating the damage profile of the striated muscle cells. Partial decomposition of the cell contents resulting in the development of electron optically empty spaces (arrow) within the cortical cytoplasm under the intact plasma membrane of the muscle cell (M); glycogen (G); microtubuli (arrowhead). Bar = 1 μm.

filled all requirements not only for photoablation but also for optical breakdown. The latter is obtained by an avalanching effect of ionization through free electrons in a plasma state caused by energy concentration above 10^9 J/cm^2 in a laser beam with a pulse duration no more than 3 μs (27). In short, a plasma is a "sea" of free electrons and positive ions. Energy concentrations such as those generated in our settings have not been reported by other authors for holmium:YAG laser beams, especially for holmium:YSGG lasers transmitted through silica optical fibers [20, 28].

The holmium:YAG laser has a high absorption coefficient resulting in limited depth of tissue penetration and therefore lacks the hemostatic quality of a Nd:YAG, which seems to make it unsuitable for extended surgical procedures such as partial glossectomies. It does, however, lead to some tissue coagulation, on which hemostasis is dependent, and is thus theoretically preferable for precise soft-tissue interaction such as in vocal cord or functional endoscopic sinus surgery as well as for the removal of small neoplasms that may hemorrhage. The holmium:YAG laser, which is superior to other continuous wave lasers and ready for clinical use, is being tested for flexible microendoscopic laser surgery in partially or completely obliterated cochleas for intracochlear placement of the stimulation electrode of cochlear implants and in middle ear surgery, especially on stapes and other ossicles. Potential risks may consist in damage to the acoustic sensor and ganglion cells in the inner ear and to the spiral ligament outside the primary target area of the laser due to the sudden material ejection caused by the "blast effect" associated with photoacoustic phenomena. This "blast effect" furthermore may prove a handicap for microendoscopic delivery by means of endoscopes with a working tunnel, allowing improved visualization and higher accuracy with less risk of inadvertent injury to adjacent structures, since the particles ejected after every single laser shot cause soiling of the optical system.

Temperature measurements taken by our study group in pilot studies indicate that during the laser pulse only little heat is lost to the surrounding tissue by thermal diffusion [29]. The maximum temperature increase in the mucosa of the live animals following holmium:YAG laser exposure (400 mJ, 2.5 μs, 5 Hz) was moderate. During 10 seconds of laser exposure, the temperature rose from an initial 32°C, measured directly in the tongue at a distance of 800 μm from the laser target zone, to 58°C. This is in agreement with our micromorphological examinations of holmium:YAG laser effects, which show no signs of a thermic melting process or of carbonization. This in turn is in contrast with the findings of previous ultrastructural studies undertaken by our group with the purely thermal CO_2 laser, where three zones of damage with an inner carbonization zone were found [30]. In addition, the observation of partial decomposition of the cell contents in muscle cells resulting in the development of electron optically empty spaces within the cortical cytoplasm underneath the intact plasma membrane is likely to be a photoablative or photoacoustic effect. The absence of tiny cracks within the cell membrane, to be expected as a result of photothermal laser-induced vapor escape after the boiling point of water is reached within the cell, and the fact that the organelles within the cell remain ultrastructurally intact fit the assumption of an additional nonthermal holmium:YAG laser-tissue interaction.

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